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Article

The effects of metformın, acetylsalıcylıc acıd and ıbuprofen on telomerase enzyme actıvıty: ınhıbıtory effect of ıbuprofen

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Telomerase enzyme is necessary for the elongation of telomeres while telomerase being critical for aging and cancer. Metformin, ibuprofen, and acetylsalicylic acid used in this research are drugs that millions of people already use and that many are likely to use in future. In this study, the effects of these drugs on telomerase activity of Mus musculus swiss albino mice in liver tissue were investigated and the telomerase activity was measured with a PCR- ELISA based kit. In the study a possible connection between telomerase enzyme activity and activities of antioxidant enzymes was also investigated by determining the activity of superoxide dismutase (SOD) and catalase enzymes. The data obtained show that metformin slightly decreased telomerase enzyme activity in low dose application; however, this change was not statistically significant. In ibuprofen application, there was a significant inhibitory effect when high doses were used; whereas, there was a slight inhibitory effect at low doses. In acetylsalicylic acid application, a slight activator effect was detected; it was not statistically significant, though. Metformin was observed to increase catalase and SOD activities in general while low and high doses of acetyl salicylic acid showed different effects. In addition, ibuprofen caused a statistically significant increase in liver SOD values. It is important to note that this study demonstrated a significant inhibitory effect of ibuprofen on telomerase enzyme activity in animal models..

Keywords: Telomerase. Metformin. Ibuprofen. Acetylsalicylic acid. SOD. Catalase.

INTRODUCTION

In the mid-1980s, Blackburn and Greider's study showed that the presence of an enzymatic activity in cell extracts that added tandem hexanucleotides to their natural chromosome ends led to the discovery of telomerase (Lu *et al*., 2013). Telomeres are added independently of normal DNA synthesis with a specific reverse transcriptase, telomerase, which contains an RNA template for the synthesis of telomeres (Figure 1). Recent studies show that telomere length plays a role in both human tumor formation and aging. Telomerase is mostly insufficient in somatic cells. Therefore, telomeres have been shown to become shorter in each replication cycle, leading to chromosome instability and aging (Rhodes, Giraldo, 1995). While telomerase activity is detected in almost all types of cancer, telomerase appears to be an almost universal target for cancer therapeutics due to the lack of effective telomerase activity in most normal human cells (Shay, Wright, 2011).

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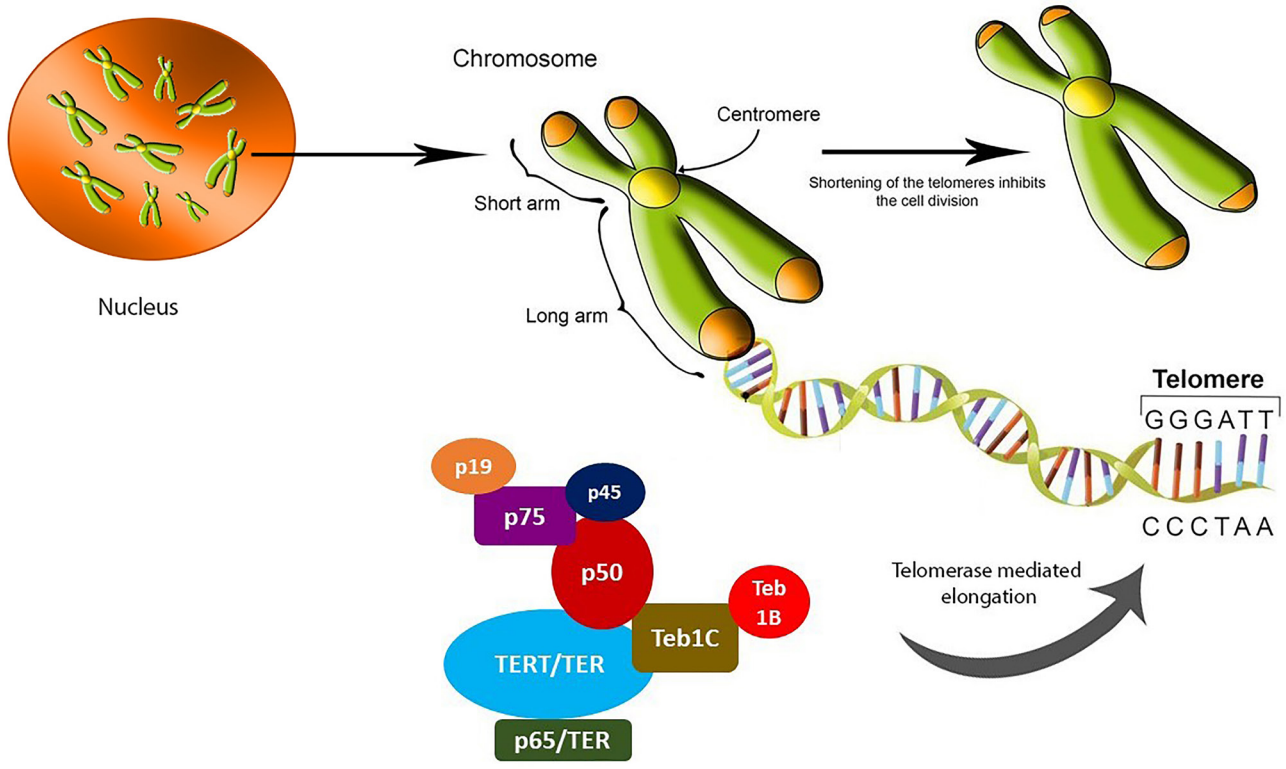

FIGURE 1 - Telomere and Telomerase (Telomerase schematic drawing modified from Jiang et al. 2013).

The aim of this study is to determine the potential activatory or inhibitory effects of metformin, ibuprofen and acetylsalicylic acid on telomerase enzyme. In addition, it has been investigated whether there is a link between telomerase activity and antioxidant enzyme activities.

Metformin, ibuprofen and acetylsalicylic acid are drugs used by millions of people. The number of people who use these drugs for life or at a certain period of their lives is very high. According to the ClinCalc Drug statistics 2021 database, in 2018, metformin-containing drugs were prescribed 3,991,205 times to 727,627 patients, Ibuprofen-containing drugs were prescribed 24,453,501 times to 11,636,681 patients, and aspirin was prescribed 19,750,180 times to 5,004,736 patients. This intensity of use makes it important to know the other possible effects of these drugs. The reason for choosing these drugs in this specific study is related to the available information in the literature concerning all three have effects on cancer or life span. Since the telomerase is an enzyme that is effective on both cancer and life span, it is thought to be useful to investigate whether some of the effects of these drugs mentioned in the literature are due to telomerase.

Metformin, an important drug for the treatment of type 2 diabetes for many years; is the most commonly used oral antihyperglycemic agent and is still recommended as first-line therapy for all newly diagnosed T2D patients as it alleviates hyperglycemia without causing hypoglycemia or weight gain and without damaging the cardiovascular system (Foretz *et al.*, 2014). Studies show that metformin has anticarcinogenic properties and its use is associated with positive results in many types of cancer such as breast cancer, ovarian cancer, colon cancer and prostate cancer (Wang *et al.*, 2015; Patel, et al., 2015). The mechanisms that provide the inhibitory effects of metformin on tumor development are not fully understood but are thought to have indirect effects on systemic insulin or glucose levels or effects on tumor cell growth and survival. In studies that have been

shown to increase cell death, metformin appears to be associated with the activation of the apoptotic pathways (Zhuang, Miskimins, 2011).

Ibuprofen is a drug from the group of NSAIDs that is also used to treat mild to moderate pain and inflammation and to reduce fever (Bramlage, Goldis, 2008). Ibuprofen, which is included in the "List of Essential Medicines" of the World Health Organization, is one of the drugs produced in large amounts worldwide (Han *et al.*, 2010).

Acetylsalicylic acid as a new drug was first synthesized in 1897 by Felix Hoffmann. Hoffmann and tested on tested on patients the following year. Results from clinical and animal experiments were published in 1899, and in 1904, the powder form was converted into tablets, which led to its increased daily use. Since then, aspirin has become the most widely used drug worldwide (Pasche *et al.*, 2014). Aspirin is a common non-steroidal anti-inflammatory drug that is often used for analgesia or as an antiplatelet agent in low dose myocardial infarction and stroke prevention and is also thought to have chemo-protective or even therapeutic properties for many common types of cancer (Fraser *et al*., 2014).

Reactive oxygen derivatives can be produced in some natural processes inside the cell and there is an increase in its production due to the influence of some external factors as well. These oxygen radicals are neutralized by the body's antioxidant defense system. However, if there is more radical production than the antioxidant system can handle, the balance is disturbed and oxidative stress occurs. The relationship of oxidative stress with aging and some diseases has been known for a long time (Liguori *et al.*, 2018). It has been suggested that exposure to oxidative stress causes shortening of telomeres, and a decrease in stress can provide protection of telomeres (Kim *et al.*, 2016). In their reviews, Reichert and Stier (2017) stated that most studies on this topic in the related literature showed a possible link between telomere shortening and oxidative stress; however, the data obtained from the studies on this issue were not sufficient.

In this study, a link was also investigated between telomerase and antioxidant enzymes by measuring the activity of SOD and catalase enzymes as well as telomerase enzyme activity.

MATERIAL AND METHODS

Experimental Design, Mouse Care and Applications

Mus musculus Swiss albino mice were used in this study (Burdur Mehmet Akif Ersoy University animal experiments ethics committee, decision dated 15.08.2018, numbered 390).

Ibuprofen, metformin, and acetylsalicylic acid were applied to 2.5-4.5 month old adult female mice after 7 days of acclimation period. The animals were housed in plastic cages on sawdust bedding, and fed with standart diet and tap water ad libitum. The mice were housed and treated in accordance with national and institutional guidelines. The room temperature was a 23 ± 2◦C, with relative humidity of 45%, and a 12h daynight-light cycle.The dose of metformin to be given to the mice was 32 mg/kg and 13 mg/kg. Ibuprofen and acetylsalicylic acid were given as 10 mg/kg and 2 mg/kg. The solutions were prepared in DMSO in order that this doses could be given given to the mice in 10 µL of liquid and the agents were administered to the mice by automatic pipette for 30 days. Experiments were carried out with six mice in each experimental group and at the end of the 30-day application period, the mice were exposed to ether anesthesia and dissected, so that liver and kidney tissues could be taken. The reason for using liver tissues is that the highest telomerase activity in mice is found in liver and testicular tissues (Prowse, Greider, 1995). The sample size for each treatment group was determined by considering the statistically necessary number of subjects in the case of using mice as experimental animals and the ethical rules in the use of experimental animals.

While determining the doses of the substances applied in this study, attention was paid to select the doses that people could be exposed to in daily life, while very high doses were not preferred for treatment purposes.

Measurement of Enzyme Activities

Roche Telomerase PCR-ELISA kit was used to measure telomerase activity and SIGMA 19160 SOD assay kit was used to measure SOD activity. Applications were carried out according to Luck's method (Luck, 1963) based on measuring the breakdown of H_2O_2 by catalase in tissue extract and the decrease in absorbance at 240 nm.

Protein determination was performed on tissue extracts using the Lowry method (Lowry, 1951). Non-parametric tests were used for statistical analysis using the minitab program.

RESULTS AND DISCUSSION

The mean of Relative Telomerase Activity (RTA) values in the application groups are as shown in Table I. It was determined that Ibuprofen High Dose group showed statistically significant ($p < 0.05$) inhibitory effect.

TABLE I - Relative telomerase activity results obtained from application and control groups (Metformin H 32 mg/kg; Metformin L 13 mg/kg; Ibuprofen H 10 mg/kg; Ibuprofen L 2 mg/kg; Acetylsalicylic acid H 10 mg/kg; Acetylsalicylic acid L 2 mg/kg)

Groups	RTA Values ± SE
Metformin H	35.45 ± 2.23
Metformin L	33.55 ± 2.78
Ibuprofen H	29.30± 1.87*
Ibuprofen L	31.14 ± 1.27
Acetylsalicylic acid H	39.80 ± 1.88
Acetylsalicylic acid L	38.96 ± 1.79
Control/Vehicle Group (DMSO)	36.90 ± 2.86

* Statistically different from control ($p < 0.05$)

According to antioxidant enzyme analyzes (Table II), both low and high doses of metformin provided a significant increase in liver SOD activity compared to that of the control while a general increase in both liver and kidney SOD and Catalase activities was observed.

TABLE II - SOD and Catalase Enzyme Activity Results (Metformin H 32 mg/kg; Metformin L 13 mg/kg; Ibuprofen H 10 mg/kg; Ibuprofen L 2 mg/kg; Acetylsalicylic acid H 10 mg/kg; Acetylsalicylic acid L 2 mg/kg)

GROUPS	CAT Kidney	CAT Liver	SOD Kidney	SOD Liver
MF H	5222.9 ± 438	1617.4 ± 276.7	288.3 ± 26.2	120.1 ± 7.7$^{\Delta}$
MF L	5618 ± 491.7	1506.6 ± 147.7	315 ± 32	114.7 ± 5.8$^{\Delta}$
ASA H	4626.8 ± 308.1*	1393.1 ± 192.8	259.3 ± 22.2	109.3 ± 17.5
ASA L	3358 ± 459.7$^{\Delta}$	1471.3 ± 247.1	255.9 ± 17.6	88.7 ± 15.8
IBP H	4143.6 ± 280.5	1504.1 ± 174.4	245.6 ± 13.2	117.3 ± 16.5$^{\Delta}$
IBP L	5166.5 ± 533.2	1781.3 ± 310.5	296.2 ± 28.2	121.2 ± 15.9$^{\Delta}$
DMSO (Vehicle)	5148.3 ± 478.3	1166.6 ± 208.8	288.2 ± 25.6	76.6 ± 5.5

* Statistically different from low dose ($p < 0.05$)
$^{\Delta}$ Statistically different from control/vehicle ($p < 0.05$)

Low-dose Acetylsalicylic acid reduced antioxidant enzyme activities in the kidney when compared to its control and high dose (statistically significant in kidney) activities, whereas in the liver, it slightly increased enzyme activities.

While Ibuprofen application caused a statistically significant increase in liver SOD values, other differences were not found statistically significant.

According to the results obtained from this study, metformin did not cause any significant changes in telomerase activity in mouse liver. Although it was observed that low dose application (13 mg/kg) caused a slight decrease, this difference was not statistically significant.

In fact, most of the studies on cancer-metformin relationship in the literature are trials on cell culture and different results can be obtained from mouse studies since they contain differences in both their application dose and metabolism of the substance. Also, in terms of telomerase enzyme, it can be seen in the literature that any applied substance shows different results even between cancer cell lines and normal cell lines.

When we looked at antioxidant enzyme activities, we observed that metformin increased SOD and catalase activities in both doses of liver and kidney. Therefore, it can be said that metformin has an antioxidant feature.

Some studies have reported that biguanides, especially metformin, have geroprotective effects. This therapeutic profile of metformin supports its use for age-related diseases and longevity. Many studies have also confirmed the positive effects of metformin on the life span of worms, flies, mice and rats. Likewise, chronic treatment with metformin in patients with diabetes is thought to reduce the risk of cognitive decline and dementia and increase survival in various types of cancer (Novelle *et al*., 2019). Publications showing a decrease in cancer and cardiovascular disease risk in patients treated with metformin are constantly on the increase (Anisimov, 2013).

Studies related to telomerase have generally focused on the effect of metformin on hTERT expression. In a study by Cantrell *et al.* (2010), metformin caused decrease in hTERT expression and induction of apoptosis. In addition, the combination of metformin and metformin - paclitaxel reduced hTERT mRNA expression, while paclitaxel alone had no effect on telomerase activity (Hanna *et al*., 2012).

According to the results obtained from our study, a higher dose of ibuprofen administered as 10 mg/kg showed a statistically significant inhibition of telomerase in the mouse liver. In fact, there is also a slight inhibition at a dose of 2 mg/kg, however inhibition is not statistically significant. In this case, it may be thought that some of the effects of ibuprofen reported in the literature, especially anti-carcinogenicity, may also be associated with the dose dependent inhibitory effect of the telomerase enzyme.

In addition, ıbuprofen decreased catalase and SOD activities in the kidney and increased it in the liver. While the antioxidant effect of ibuprofen is more pronounced at low doses, it seems that this effect persists partially for the liver at high doses, but high doses may have prooxidant effects in the kidney.

The related literature shows that ibuprofen can effectively suppress proliferation and induce apoptosis of prostate cancer cells at clinically relevant concentrations (Andrews *et al*., 2002). In addition, ibuprofen therapy is effective on gastric cancer cell proliferation. Ibuprofen has been shown to significantly inhibit the proliferation of Gastric cancer cell line MKN-45 cells based on time and concentration (Bonelli *et al*., 2011). Some clinical, laboratory, and animal studies have shown that some other nonsteroidal anti-inflammatory drugs (NSAIDs) clinically used in the treatment of pain and inflammation also have anticarcinogenic effects in colon cancer (Redpath *et al*., 2009).

There are very few studies on the effect of ibuprofen on telomerase activity and these studies were performed generally in a cell culture environment. In a study on cultured MCG-101 (Murine sarcoma) cells, it was observed that all NSAIDs except ibuprofen depress telomerase activity (Lonnroth, Andersson, Lundholm, 2001). In addition, it was observed that the ibuprofen did not affect telomere length in peripheral blood and skeletal muscle cells. (Ekstrand, 2011).

According to our findings, acetylsalicylic acid slightly increased telomerase activity in the mouse liver. However, this increase was not found statistically significant. As is discussed in this paper, the results in the literature show differences depending on the type of cell applied. However, the increase in telomerase activity we found may be considered to be compatible with the antioxidant effect of acetylsalicylic acid, which is mentioned extensively in the literature. In our specific study, acetyl salicylic acid appears to play an antioxidant role in the liver and a prooxidant in the kidney.

Aspirin is an antipyretic analgesic with a long history can be used to treat colds, fever, headache, toothache, joint pain, and rheumatism. It can also inhibit platelet aggregation, prevent and treat ischemic heart disease, cardiopulmonary infarction, cerebral thrombosis and other diseases. In addition, acetylsalicylic acid has been shown in some studies to have an anti-cancer effect. It has been suggested that acetylsalicylic acid can be used to prevent colorectal cancer in groups at high risk for colorectal cancer. It has also been shown that daily intake of a certain dose of acetylsalicylic acid can effectively prevent the growth of breast cancer. In addition, acetylsalicylic acid can have a certain inhibitory effect on colon cancer, gastrointestinal cancer, prostate cancer and other cancers (Huang *et al*., 2018).

Although these above mentioned effects of aspirin are in the literature, there are few studies on how it affects telomerase enzyme activity. When endothelial cell culture is treated with aspirin, telomerase activity has been shown to increase (Bode-Böger *et al*., 2005). Another study conducted by Li et al., 2013 revealed that aspirin inhibits telomerase activity in Polymorphonuclear neutrophils (PMN) from carotid lipid-rich plaques, it had no inhibitory effect on circulating PMNs, however.

In a study by Hua He *et al.* (2006), how NSAIDs affect telomerase activity in colon cancer cell lines it was observed that aspirin, indomethacin and SC-236 inhibit telomerase activity at HT-29, COLO205 and CRL-2134 at different levels compared to control cells treated with DMSO, but there was no significant inhibitory effect on telomerase activity in SW1116 cells.

In other words, changes in telomerase enzyme activity generally differ depending on the cell line used or whether the cells are cancerous or normal cells. This current study also contributes to the literature in terms of conducting the study.

As a result, according to the data obtained in our study, although metformin slightly decreased the activity of telomerase enzyme on low doses, this change was not statistically significant. In ibuprofen application, there is a significant inhibitory effect when high doses are used, and a slightly inhibitory effect at low doses. In acetylsalicylic acid application, a slight activator effect was detected, although not statistically significant.

It is also important to note in this study that ibuprofen had a significant inhibitory effect on telomerase enzyme activity in animal models.

Moreover, metformin was observed to increase catalase and SOD activities in general while low and high doses of acetyl salicylic acid showed different effects. Ibuprofen caused a statistically significant increase in liver SOD values as well.

However, there was no clear correlation between the effects of these substances on telomerase enzyme activity and their effects on antioxidant enzyme activity.

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